

Comparing Alternative Approaches to Establishing Regulatory Levels for Reproductive Toxicants: DBCP As a Case Study

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This paper compares four alternative approaches for deriving regulatory levels for reproductive toxicants by applying them to the available data on the human spermatotoxicant 1,2-dibromo-3-chloropropane (DBCP). The alternatives examined include the Proposition 65 approach (application of a mandatory 1000-fold uncertainty factor to a no-observed-adverse-effect level [NOAEL]), the Environmental Protection Agency (EPA) approach (application of flexible uncertainty factors to a NOAEL), the Benchmark Dose approach (application of flexible uncertainty factors to a dose associated with a known level of change in a reproductive parameter), and the Quantitative Risk Estimation approach (using low-dose linear extrapolation and a model of the relationship between sperm count and infertility).

Applied to DBCP, these approaches do not produce substantially different estimates of allowable exposure levels. However, the approaches do have different data requirements and provide different amounts of information on reproductive hazards to risk managers and the public. Neither the Proposition 65 nor the EPA approach provides information about the extent of health risk remaining at a regulatory level. In contrast, the Benchmark Dose approach can provide estimates of the magnitude of sperm count reduction at a regulatory level, and the Quantitative Risk Estimation approach can provide estimates of exposure-induced infertility.

Introduction

California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) represents the first generic effort to regulate human exposures to reproductive toxicants. Its passage reflects the widespread public concern that environmental exposures may be a significant and avoidable cause of reproductive or developmental harm. As of April 1990, 63 substances were listed as chemicals known to the State to cause reproductive toxicity. The need to establish regulatory levels for these substances provides an opportunity to compare several approaches to calculating acceptable levels of exposure to reproductive toxicants.

Proposition 65 adopts the conventional regulatory

approach to noncarcinogenic end points. A no-observed-adverse-effect level (NOAEL) for reproductive or developmental toxicity is identified and divided by an uncertainty factor (UF) to establish an allowable exposure level. Believing that the common use of uncertainty factors of 100 or less did not provide sufficient protection, supporters of Proposition 65 required the use of an uncertainty factor of 1000. Due in part to the perceived inflexibility of this statutory mandate, no regulatory levels for listed chemicals have been derived using conventional risk assessment methods. Proposals to amend the reproductive toxicity sections of Proposition 65 have been debated in the California Legislature and provide the political context for a comparison of alternative approaches.

Both in California and at the Federal level, regulatory agencies have been encouraged to develop alternatives to the conventional NOAEL/UF approach to regulating reproductive and developmental risks (1,2). The NOAEL/UF approach is limited because it does not make use of all available dose-response data and cannot provide an estimate of the reproductive risk associated with a regulatory level. Several reviews of recent efforts to develop risk estimation procedures for

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reproductive and developmental toxicants have been published (3,4).

The goal of any risk assessment approach is to provide a scientifically defensible derivation of a regulatory level or "reference dose." Following the usage of the Environmental Protection Agency (5), a reference dose (RfD) is defined as an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfDs discussed throughout this paper are based on male reproductive toxicity.

Different approaches to deriving reference doses have different data requirements and provide different amounts of information on reproductive hazards to risk managers and the public. This paper compares four different approaches to deriving an RfD in an effort to characterize their strengths and weaknesses. The four approaches examined are:

a) Proposition 65 approach. Following the requirements of Proposition 65 and procedures specified by the California Health and Welfare Agency, a NOAEL is identified and divided by a 1000-fold UF to calculate an RfD.

b) Environmental Protection Agency (EPA) approach. Following the practice of EPA, an NOAEL is identified and divided by a UF that reflects the extent of uncertainty in the scientific evidence to calculate an RfD.

c) Benchmark Dose approach. As an alternative to the EPA procedure, the NOAEL is replaced by a "benchmark dose" (BD), defined as the lower 95% confidence bound on the dose associated with a 10% change in effect from the control group (6). To calculate an RfD, the BD is divided by a UF that reflects the extent of uncertainty in the scientific evidence.

d) Quantitative Risk Estimation approach. Following procedures analogous to those used in cancer risk assessment, risks of an adverse reproductive effect are calculated using low-dose extrapolation to estimate the relationship between 1,2-dibromo-3-chloropropane (DBCP) exposure and sperm count reduction and a two-distribution model (7) to predict the relationship between sperm count reduction and risk of infertility. Estimates of maximum individual and aggregate population risks may be used to establish acceptable levels of reproductive risk.

The agricultural nematocide DBCP has been selected to illustrate these approaches because of the availability of dose-response data and the need to derive a regulatory level for this chemical under Proposition 65. DBCP is a known human reproductive toxicant, causing decreased sperm counts and increased incidence of infertility. Under Proposition 65, DBCP has been listed as a chemical known to the State to cause reproductive toxicity and cancer. After reviewing background information on DBCP and its toxicology, factors needed to assess reproductive risk are discussed, regulatory levels are derived using the four approaches, and these alternatives are compared.

DBCP: A Case Study

Exposure to DBCP in California

Widespread use of DBCP has resulted in the contamination of a number of aquifers in California (8). DBCP has been detected in 2113 of 5288 wells sampled; these wells are located in 15 of 26 counties surveyed in California (9). Since the median half-life of DBCP in groundwater is approximately 20 years (10), DBCP may persist in California aquifers at detectable levels for over a century.

Contaminated groundwater is currently the major source of exposure to DBCP in California; exposure to DBCP through food and ambient air has been insignificant since most agricultural use of DBCP was banned by EPA in 1977. Approximately 200,000 Californians are exposed to DBCP in water provided by large public systems in the Central Valley; the number exposed through private wells is unknown. There are three pathways to be considered in assessing total personal exposure to DBCP from domestic water: ingestion of DBCP-contaminated water, inhalation of DBCP volatilized into indoor air from daily water use, and dermal absorption from bathing. About one-third of an individual's total DBCP dose is derived from each pathway (11,12). The average dose per exposed person ranges between 2 and 4×10^{-5} mg/kg-day (11).

Reproductive Toxicity of DBCP

The adverse effects of DBCP on human testicular function became widely recognized with the 1977 discovery of azoospermia or severe oligospermia in 14 of 25 male DBCP production workers at an Occidental Petroleum plant in Lathrop, California (13). These findings were confirmed by a later extensive study at the same plant. For the 107 workers who were ever exposed to DBCP, the median sperm count was 46 million/mL of seminal fluid. For the 35 workers who were never exposed, the median count was 79 million/mL of seminal fluid. Of the exposed workers, 13% were azoospermic, 17% had sperm counts less than 20 million sperm/mL, and 16% had sperm counts between 20 and 39 million sperm/mL. Among the unexposed workers, 2.9% were azoospermic, none had sperm counts below 20 million sperm/mL, and 5.7% had counts between 20 and 39 million sperm/mL. The estimated duration of exposure was inversely related to sperm counts (14). Similar adverse reproductive effects have been reported in other studies of DBCP production workers (15-17). Figure 1 illustrates the effect that DBCP exposure had on the distribution of sperm counts in workers at a plant in Mobile, Alabama (18).

The consequence of DBCP's adverse effects on spermatogenesis include reduced fertility (subfertility) or infertility among exposed workers. Couples in which the male partner was employed at the Lathrop facility exhibited a standard fertility ratio of 0.75 for the periods when the husband was at risk of DBCP exposure,

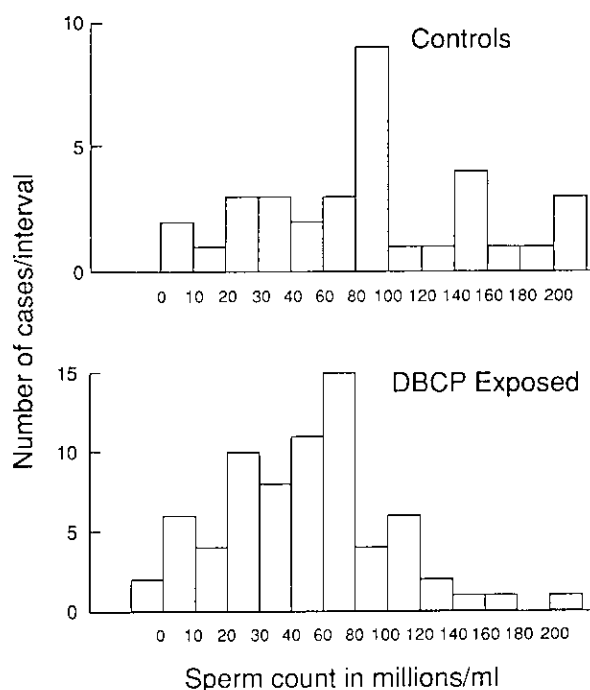


FIGURE 1. Frequency distribution of sperm counts of workers in a plant in Mobile, Alabama, manufacturing DBCP (7,18).

corresponding to a 25% decrease in fertility (19).

Male exposure to DBCP is also associated with adverse effects on the conceptus. The frequency of spontaneous abortions was significantly elevated among wives of field applicators after their husbands had exposures to DBCP (in banana plantations in Israel), compared to the frequency of spontaneous abortions before paternal exposure to DBCP (20). The male-to-female sex ratio among offspring whose fathers were exposed to DBCP was significantly lower than the ratio among offspring fathered by unexposed men (21). There were no congenital abnormalities observed among children of workers who had received sufficient DBCP exposures to induce oligospermia (22,23). Sufficient studies of DBCP's developmental effects or reproductive effects on females have not been conducted.

DBCP has multiple targets in the male reproductive system, including stem cells, epididymal cells, and postmeiotic cells, and its mechanism of action at each target may well be different. In humans, spermatogonial stem cells appear to be most susceptible to DBCP toxicity. Histopathological examination of testicular biopsies from DBCP-exposed workers revealed degeneration of the germ cells of seminiferous tubules, indicating that "suppression of spermatogenesis was related to a failure of the normal process responsible for the renewal of spermatogonia at the stem-cell level" (24). The toxicity of DBCP could be due either to direct action on the stem cells or to modifications of environmental factors critical to the maintenance of stem cells.

The direct action hypothesis is supported by studies

indicating that DBCP targets cellular DNA, initiating a process ultimately leading to organ necrosis (25). DBCP is metabolized by the cytochrome P-450 system or the glutathione conjugation system to form reactive intermediates that covalently bind to nucleophilic sites in macromolecules (26,27). Binding of DBCP metabolites to testicular cell DNA has been demonstrated (25). Other researchers have postulated that DBCP may be selectively cytotoxic to germ cells due to their dependence on aerobic metabolism (28). DBCP is known to disrupt sperm carbohydrate metabolism (29), and the inhibition of energy processes at the level of mitochondrial respiration could also account for DBCP toxicity to epididymal sperm (30).

DBCP could also modify hormonal or cellular factors critical to spermatogenesis, although this hypothesis is not well supported by available hormonal or histological data. Hormonal profiles in DBCP-exposed workers indicate that stem cells are the likely target of the compound (14,15,18,31-34). Follicle-stimulating hormone (FSH) levels, a measure of germ cell aplasia, are consistently elevated. Any disruptions of the pituitary/hypothalamic axis appear to be secondary to DBCP's effect on the testis (35). Testosterone levels, a measure of Leydig cell function, were generally normal, except in severely affected workers who showed no sign of recovery from DBCP exposure (17,36). This indicates that DBCP does not primarily impact Leydig cells. Histological examinations of exposed workers have determined that Sertoli cells, the other major cell type in the testis, do not appear to be affected by DBCP (24,37,38).

DBCP produces similar adverse reproductive effects (testicular toxicity, decreased fertility) in a number of mammalian test species (rats, guinea pigs, and rabbits). Reviews of acute, subchronic, and chronic studies assessing the reproductive toxicity of DBCP are provided by Reed et al. (11), the National Academy of Sciences (39), Jackson et al. (40), and Barlow and Sullivan (41). These studies demonstrate a dose-response relationship between the degree and duration of exposure and the severity of testicular toxicity. The rabbit is the most sensitive test species (42).

An inhalation dose-response study of the gonadotoxic effects of DBCP has been conducted in rabbits by Rao et al. (43). Male rabbits ($n = 40$) were exposed to DBCP in vapor at 0, 0.1, 1.0, or 10 ppm for 6 hr/day, 5 days/week for up to 14 weeks. These doses are equivalent to 0, 0.027, 0.27, and 2.7 mg/kg-day, respectively, assuming that the absorbed fraction of inhaled DBCP is 50% (11). This 50% absorption factor is based on animal inhalation studies of three low-molecular-weight, halogenated, aliphatic compounds (44). The time of onset and extent of decrease in sperm count were dose dependent, and sperm count and viability were significantly decreased in a dose-related manner at exposures of 1 ppm DBCP and higher. In the 0.1-ppm dose group, there was a temporary statistically significant decrease in sperm count at 12 weeks and an equivocal increase in sperm with abnormal morphology at 14

weeks. It is not known whether more substantial changes would have occurred with exposures longer than 14 weeks. Exposure at the 10 ppm dose caused severe atrophy of the testes, complete absence of spermatogenesis, and sterility. Exposure in the 10-ppm dose group was terminated after 8 weeks because of high mortality.

The effect of DBCP ingestion on rabbits has been investigated in studies of shorter duration (45,46), which may have limited study sensitivity. No evidence of reproductive toxicity was reported at a dose level of 0.94 mg/kg-day after 10 weeks. In the rabbit inhalation study, a significant decrease in sperm count in the 1-ppm dose group (absorbing approximately 0.3 mg/kg-day) was not observed until the end of week 11 of exposure (43). In experiments to identify male reproductive toxicants, protocols generally recommend treatment for a duration equivalent to at least six cycles of the seminiferous epithelium: 64 days for rabbits (47). The Rao et al. study (43) indicates that this period is insufficient to observe the effects of mid-range doses of DBCP on spermatogenesis, and longer exposures may be required to examine the effect of cumulative damage to spermatogonia on sperm counts.

Factors Used to Assess Reproductive Risks of DBCP

End Point Selection

Several semen parameters serve as biomarkers of reproductive toxicity in males, including sperm concentration, motility and morphology, and the volume and characteristics of semen. Decrements or changes in these parameters may represent a decrease in fertility status. Sperm count is a useful indicator of fertility status, although sperm concentration in the ejaculate may vary considerably between individuals, as well as across time in an individual. Approximately 10% of men in the general population are oligospermic, with sperm concentrations of less than 20 million sperm/mL semen (48). About 1% of men have essentially no sperm and are azospermic. Sperm motility and morphology are less variable in an individual and can also be used to evaluate changes in fertility (49). A relationship between human male infertility and the proportion of abnormally shaped or poorly motile cells in the ejaculate has been shown (50). In the case of DBCP, sperm count provides the best measure of reproductive toxicity that has been quantitatively ascertained in humans and animals.

NOAEL Identification and Selection

Although there is sufficient evidence in humans that DBCP adversely affects reproductive function in males, the dose-response data from these epidemiological studies are not adequate for risk assessment purposes and cannot be used to determine a human

NOAEL (11,51). Exposure estimates are limited by incomplete workplace air monitoring data and by the unknown contribution of dermal absorption to the overall DBCP exposure. The exposure history of workers prior to DBCP exposure is also unknown. Exposed workers experienced a range of adverse effects; data are insufficient to assign specific percentage decreases in sperm counts to particular DBCP dose levels.

Since the data in humans are unreliable, reference doses for reproductive toxicity are derived using the inhalation NOAEL for rabbits described in the study by Rao et al. (43). This study was selected because it employed the most sensitive test species and was well conducted. However, variability in the data makes it difficult to determine whether the study actually identified a NOAEL. In the 0.1-ppm dose group, both a temporary decrease in sperm count and a nonsignificant increase in the percent of abnormal sperm were observed. After examining the data using different statistical techniques, we have accepted the conclusion of Rao et al. that the NOAEL was 0.1 ppm under the conditions of this study. The administered dose corresponding to this NOAEL is 0.054 mg/kg-day, and the estimated absorbed dose is 0.027 mg/kg-day (11).

The decision to use animal data instead of human data for risk assessment purposes raises an important issue. Human males may be more vulnerable to the effects of reproductive toxicants than other mammals (52) because the production of human sperm per gram of tissue is one-fourth that of commonly used laboratory animals (53). In addition, the ejaculate of fertile men contains a much higher proportion of abnormally shaped sperm than the ejaculate from laboratory animals (54). Thus, exposures that produce similar (absolute or percent) changes in humans and experimental animals in either sperm count or frequency of abnormal sperm may decrease male fertility to a greater extent in humans than in animals.

The decision to select a NOAEL from an inhalation rather than an ingestion study requires discussion. There is no direct information on reproductive effects in humans resulting from oral exposures to DBCP. The potency of DBCP as a reproductive toxicant may be influenced by route-specific rates of absorption and activation/detoxification. In rabbits, the most sensitive experimental species, DBCP was a more potent spermatotoxicant by inhalation than by ingestion: the NOAEL for reproductive effects from DBCP by inhalation was 0.027 mg/kg-day (assuming 50% absorption), and the NOAEL from DBCP by ingestion was 0.94 mg/kg-day (assuming 100% absorption) (11). This difference in NOAELs may be due to the difference in exposure period or to differences in the pharmacokinetics of ingestion and inhalation exposures. Regulatory levels should be based on the most sensitive study because pharmacokinetic data are not available to estimate the effective doses that are produced by different routes of administration, and inhalation as well as ingestion exposures to DBCP occur with contaminated drinking water.

Estimating Reference Doses and Reproductive Risks for DBCP: Four Alternative Approaches

Proposition 65: Use of a NOAEL and an Uncertainty Factor of 1000

Procedures for calculating a daily intake level for a reproductive toxicant are specified in Proposition 65 [California Health and Safety Code Section 25249.10(c)] and its implementing regulations (Title 22 California Code of Regulations Section 12803). An RfD for reproductive toxicity is calculated according to the formula:

$$\text{RfD} = (\text{NOAEL} \times \text{reference body weight})/1000,$$

where the NOAEL is determined for the reproductive effect that formed the basis of the chemical's listing under Proposition 65. The NOAEL is to be taken from the most sensitive study deemed to be of sufficient quality. The reference body weight for an adult male is 70 kg and an uncertainty factor of 1000 is required by Proposition 65.

Using the NOAEL (0.027 mg/kg-day absorbed dose) from the rabbit inhalation study by Rao et al. (43), the RfD for DBCP calculated using Proposition 65 is

$$\begin{aligned} \text{RfD} &= (0.027 \text{ mg/kg-day} \times 70 \text{ kg})/1000 \\ &= 1.9 \times 10^{-3} \text{ mg/day, or approximately} \\ &\quad 2 \text{ } \mu\text{g/day} \end{aligned}$$

Over 69,000 Californians in the Central Valley using large public water systems as sources of drinking water receive average daily DBCP doses exceeding 1.9 μg . The maximum daily DBCP exposure experienced by about 6,000 people is estimated to be 3.0×10^{-4} mg/kg, or about 21 μg . Estimates of the number of exposed persons are likely to be low because the extent of DBCP contamination of private wells used for drinking water is unknown (11).

EPA: Use of a NOAEL and an Uncertainty Factor Based on the Extent of Uncertainty in Scientific Evidence

State and Federal regulatory agencies commonly apply uncertainty factors of 100 or more to animal data when establishing allowable environmental exposure levels (55). Generally, State and Federal regulatory agencies combine several factors to account for various sources of uncertainty in the risk assessment process: a factor of 10 to reflect variation in sensitivity within the human population; a factor of 10 to reflect uncertainties in extrapolation from animals to humans; a factor of 10 to reflect extrapolation from subchronic study results to chronic exposure conditions; a factor of 10 when a lowest-observed-adverse-effect level (LOAEL) is used rather than a NOAEL; and a modifying factor, ranging from greater than 0 to less than or equal to 10, to reflect other uncertainties in the studies such as the number of species tested and the completeness of the

overall data base (5,56).

The assignment of values of 10 to the factors is arbitrary, but data have been developed that lend support for their use (55,57,58). The minimum uncertainty factor used with human data is 10, to account for person-to-person variability in the population. The minimum uncertainty factor used with animal data is 100, to account for the variability in the human population and for the uncertainties of animal-to-human extrapolation.

Selection of uncertainty factors must be made on a case-by-case examination of a toxicant's biology and exposure scenario. In the case of DBCP, a judgment must be made about whether the 14-week period of exposure employed by Rao et al. (43) constitutes a subchronic or chronic study. Administration of a test compound over one complete cycle of the seminiferous epithelium is recommended in reproductive toxicity guidelines so that the potential for an agent to produce an effect at any point in the spermatogenic process can be detected. Depending on a toxicant's mechanism of action, longer exposures than those recommended could result in progressive damage and additional adverse effects.

The design of the Rao study meets these guidelines: rabbits were exposed for 98 days, significantly longer than the 64 days required for a complete cycle of seminiferous epithelium. However, the study results strongly suggest that DBCP's effect on sperm count is due to accumulated damage among stem cells. Reductions in count only become evident in weeks 8 to 10, indicating "that DBCP primarily affected spermatogonia" and not later stages of sperm development (43). If the Rao study had been terminated after 10 weeks, satisfying the guidelines, its NOAEL would have been 1 ppm. After 14 weeks of exposure, significant sperm count reductions in the 1-ppm dose group were evident, and the NOAEL identified was one order of magnitude lower, 0.1 ppm. The ability of the stem cell population to generate sufficient sperm for successful reproduction is clearly reduced with longer exposures to DBCP in humans (14). The probability of recovery from DBCP-associated sperm count reductions is dependent on cumulative dose. A reasonable explanation for these observations is that the stem cell population is depleted over time by DBCP toxicity and exhibits decreased capacity to repopulate itself and produce new sperm.

The relevant human exposure scenario for DBCP involves continuous ingestion, inhalation, and dermal exposures from the use of contaminated groundwater. Thus, risk assessment must consider cumulative lifetime exposure to DBCP. All of the available animal data are derived from subchronic experiments (10–14 weeks). The effects of lifetime exposures to DBCP on reproductive function have not been studied in animals. Use of an uncertainty factor to account for the short duration of the animal study is appropriate because DBCP's effect on sperm count appears to increase over time due to the cumulative impact of toxicity to stem cells.

In the case of DBCP, uncertainty factors have been selected to reflect the quality of the data: a 10-fold factor for intraspecies variability, a 10-fold factor for interspecies extrapolation, a 10-fold factor for use of a subchronic NOAEL, and a modifying factor of 1 for relatively high quality data.

Selection of these factors yields an uncertainty factor of 1000, identical to the uncertainty factor mandated under Proposition 65. Using the NOAEL (0.027 mg/kg-day absorbed dose) from the rabbit inhalation study by Rao et al. (43), the RfD for DBCP derived following EPA's procedure is 1.9×10^{-3} mg/day, the same as the RfD derived using the Proposition 65 approach.

Benchmark Dose: Use of a Benchmark Dose and an Uncertainty Factor Based on the Extent of Uncertainty in Scientific Evidence

Background. The Proposition 65 and EPA approaches follow conventional regulatory practice by dividing a NOAEL by factors selected to reflect data uncertainties to calculate an RfD. Use of the NOAEL as the starting point for deriving an RfD has significant limitations (6,59). The NOAEL identified in a study may be influenced by the sample size in the study, the selection of the dose levels and their position on the dose-response curve, and the background frequency of the end point evaluated (60). Poorer studies tend to produce higher estimates of the NOAEL, which may result in less stringent regulatory levels unless appropriate uncertainty and modifying factors are applied.

NOAELs derived from studies of differing design and sensitivity present different residual rates of adverse health effects. Experimentally determined NOAELs are clearly not the same as actual no-effect (or threshold) levels for the entire population (6,59,61). A study's statistical power determines the potential rate of adverse response that may be associated with a no-observed-adverse-effect level. In a well-designed study, as little as 1% of the population may be adversely affected at the dose level identified as having no effect, whereas in another, less sensitive study, the residual rate of adverse response at the NOAEL could be as high as 20% (62).

When the appropriate data are available, the NOAEL can be replaced by a benchmark dose (BD) to provide a more uniform basis for the application of uncertainty factors. The BD is defined as a statistical, lower confidence limit on a dose producing a predetermined change in response rate from background. The BD is calculated from all of the dose-response information of a study rather than from a single point, as is done for the NOAEL (6). The advantage of the BD approach is that it can provide a starting point for the derivation of regulatory levels which represents a known level of reproductive risk.

Various benchmark levels of effect have been pro-

posed: 1% (63), 5% (64), or 10% (3). These levels have been recommended because they are easily divided by uncertainty factors to estimate potential changes in reproductive parameters at lower dose levels. To provide a consistent starting point for the assessment of different compounds, actual experimental data points have not been selected as BD levels. Extrapolations from the observed data range to a 1 to 10% effect level are not particularly sensitive to the choice of mathematical model and do not display the wide variability in risk estimates produced by different models in low-dose cancer risk assessment (6).

We have selected 10% as the BD level, following proposed California guidelines for reproductive risk assessment (56) and EPA suggestions (2,3). Given the need for a standardized approach to reproductive risk assessment by various regulatory agencies, it makes sense to use a consensus BD response level.

Table 1. Sperm counts of control and rabbits exposed to DBCP by inhalation (43).

Week of study	Millions of sperm/mL of semen, mean \pm SD			
	ppm DBCP			
	0	0.1	1.0	10
Pre-exposure				
-2	835 \pm 237	576 \pm 263	630 \pm 549	579 \pm 468
-1	694 \pm 452	673 \pm 416	552 \pm 312	546 \pm 198
Exposure				
1	728 \pm 280	494 \pm 281	538 \pm 276	503 \pm 255
2	671 \pm 342	521 \pm 135	373 \pm 244*	347 \pm 141
3	657 \pm 181	835 \pm 488	465 \pm 231	392 \pm 281 *
4	575 \pm 275	618 \pm 290	467 \pm 126	548 \pm 456
5	474 \pm 205	391 \pm 160	353 \pm 186	467 \pm 272
6	675 \pm 294	576 \pm 440	473 \pm 165	455 \pm 250
7	641 \pm 501	531 \pm 571	386 \pm 251	277 \pm 170 *
8*	426 \pm 167	423 \pm 232	301 \pm 167	124 \pm 254 *
9	509 \pm 255	420 \pm 122	510 \pm 122	124 \pm 275 *
10	473 \pm 206	459 \pm 284	376 \pm 288	7.2 \pm 7.0*
11	458 \pm 132	360 \pm 201	204 \pm 131*	5.4 \pm 2.0*
12	716 \pm 234	382 \pm 208*	356 \pm 210*	2.4 \pm 3.4*
13	758 \pm 502	738 \pm 376	248 \pm 217*	3.7 \pm 7.2*
14	602 \pm 282	498 \pm 499	109 \pm 160*	1.9 \pm 2.7*
Postexposure				
16	604 \pm 273	448 \pm 157	292 \pm 210	0.9 \pm 0.8*
19	396 \pm 220	500 \pm 190	199 \pm 221	0.3 \pm 0.2*
24	540 \pm 560	691 \pm 121	532 \pm 411	0.4 \pm 0.5*
26	452 \pm 133	629 \pm 115	328 \pm 180	1.8 \pm 3.5*
27	701 \pm 501	415 \pm 259	359 \pm 189	7.7 \pm 10.9*
28	327 \pm 213	409 \pm 308	542 \pm 261	5.1 \pm 9.6*
30	728 \pm 678	526 \pm 244	411 \pm 327	4.9 \pm 5.9*
32	357 \pm 202	628 \pm 24*	394 \pm 299	6.0 \pm 7.0*
34	520 \pm 467	535 \pm 235	544 \pm 301	7.5 \pm 11.9*
36	374 \pm 197	486 \pm 239	471 \pm 155	1.7 \pm 2.1*
38	667 \pm 689	497 \pm 196	382 \pm 442	39.6 \pm 73.0*
40	637 \pm 433	616 \pm 341	488 \pm 544	28.7 \pm 44.1*
42	429 \pm 284	231 \pm 181	559 \pm 587	2.0 \pm 2.5*
44	497 \pm 239	522 \pm 196	395 \pm 313	8.4 \pm 8.6*
46	637 \pm 350	583 \pm 276	430 \pm 107	27.2 \pm 37.2*

* Exposures of rabbits to 10 ppm were terminated after 8 weeks due to toxicity of the test material. For the 10-ppm males, the post-exposure period begins on week 9.

* Significantly different from the control value by Wilcoxon test, $p < 0.05$.

The BD for DBCP was defined as the dose that produces mean sperm counts in exposed animals that are 10% less than mean sperm counts in control animals (e.g., for rabbits with mean sperm counts of 600 million/mL in the control group, the BD produces mean sperm counts of 540 million/mL in the exposed group). To obtain the BD estimate, the lower 95% confidence limit on the dose corresponding to a 10% decrease in rabbit sperm count was calculated.

The BD is divided by a UF of appropriate magnitude (to reflect the quality of the data) to derive the RfD:

$$\text{RfD} = \text{BD}/\text{UF}$$

By defining the BD in this way (i.e., as a dose producing decreases in sperm count), it is possible to provide an estimate of the magnitude of sperm count reduction at regulatory levels which are derived using different uncertainty factors, assuming sperm count decreases linearly with dose at low doses. Exposures at the BD level, therefore, present quantifiable changes in sperm count, while exposures at the NOAEL present unknown risks of reproductive toxicity.

Calculation of a Reference Dose for DBCP. Decreased sperm count in the inhalation study in rabbits was identified as the most sensitive parameter of the reproductive toxicity of DBCP (43). The effects of DBCP on sperm count in rabbits are presented in Table 1.

Using the approach of Crump (6) and Kimmel and Gaylor (3), data from the Rao study have been modeled to estimate the benchmark dose of DBCP that would be associated with a 10% decrease in sperm count. Estimation of this dose is not particularly sensitive to the functional form of model selected, since the effect levels are close to those experimentally observed. A linear model was chosen because data on chemotherapeutic spermatotoxins indicate that sperm counts (logarithmically transformed) decrease linearly with increased absorbed dose (65,66). While the mechanism underlying DBCP's toxicity remains unclear, its activ-

ity as an alkylating agent and its ability to affect the DNA of stem cells indicate that a linear model is biologically plausible.

The original data from individual rabbits in the Rao 1982 study were used for the modeling (personal communication, K.S. Rao, 1988). Sperm counts were pooled for the time at which the effects of DBCP first became significant in the 1-ppm dose group (weeks 11 through 14 of the experiment). The high-dose group of 10 ppm was eliminated from the modeling because DBCP's systemic toxicity interfered with expression of its gonadotoxic effects. Half the test animals died in the high-dose group, even though exposure was suspended after only 8 weeks. Modeling conducted on all dose groups (using logarithmically transformed sperm counts) indicated that the BD estimate is increased by only a factor of two if the high dose group is included.

For each dose group (i) the observed sperm counts (Y_i) were assumed to follow the lognormal distribution $Y_i = e^{X_i}$, where X_i follows the normal distribution:

$$N[m(d_i), \sigma_i^2]$$

The function $m(d_i)$ represents the mean values of the log-transformed sperm count data at dose d_i , and σ_i^2 represents the variance in sperm count at each dose level.

Using a linear model,

$$m(d_i) = a + bd_i$$

where a and b are parameters that are estimated from the DBCP bioassay data. Maximum likelihood estimates of these parameters were calculated under the constraints that a is positive (i.e., control animals exhibit a positive sperm count) and that b is less than or equal to 0 (i.e., that exposure to DBCP decreases sperm count).

The maximum likelihood estimates of the DBCP dose-response equation are

$$m(d_i) = 6.395 - (4.738)d_i$$

The coefficients corresponding to the 95% lower confidence limit are

$$m(d_i) = 6.433 - (7.255)d_i$$

The results of this modeling for sperm count are graphed in Figure 2.

The 95% lower confidence limit on the DBCP dose associated with a 10% decrease in sperm count from control values (the benchmark dose) is 0.015 mg/kg-day. The NOAEL selected from this same rabbit inhalation bioassay (0.027 mg/kg-day) is estimated to be associated with an approximately 18% decrease in sperm count.

An RfD for DBCP can be established by applying the appropriate UF to the BD:

$$\text{RfD} = (0.015 \text{ mg/kg-day} \times 70 \text{ kg})/\text{UF}$$

As with the EPA approach, a 1000-fold UF was selected to reflect the quality of the data (i.e., a 10-fold

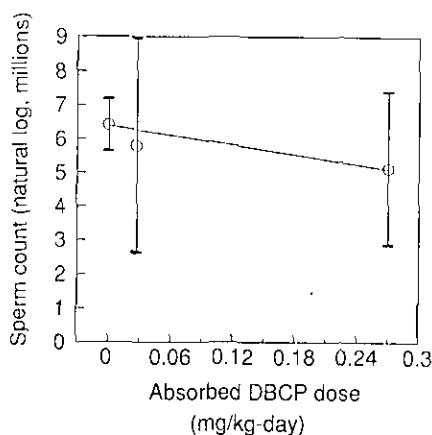


FIGURE 2. Modeled dose-response curve for DBCP effects on sperm counts in rabbits exposed by inhalation. Circles represent mean sperm count (logarithmically transformed) measured in weeks 11 through 14 by Rao et al. (43). Line is model fit.

factor for intraspecies variability, a 10-fold factor for interspecies extrapolation, a 10-fold factor for use of a subchronic NOAEL, and a modifying factor of 1). Therefore, the reproductive RfD calculated using the BD approach is 1.1 $\mu\text{g}/\text{day}$.

Estimation of Sperm Count Decrease Associated with Exposure to DBCP at the Reference Dose. The level of sperm count reduction produced by exposures to DBCP at the RfD level may be estimated using a technique proposed by Kimmel and Gaylor for estimating low-dose developmental toxicity risks (3). The level of sperm count reduction from exposure to DBCP at the RfD level (1.1 $\mu\text{g}/\text{day}$) was calculated by dividing the level of reduction present at the BD level by the UF. Exposure to DBCP at a dose that is 1000 times lower than the BD ($\text{BD}/\text{UF} = 1000$) is associated with a 0.01% decrease in sperm count (10% decrease in sperm count/1000). This procedure assumes that sperm count decreases linearly with dose and that there is no threshold in this relationship. These are conservative assumptions and should be interpreted as providing an upper bound on the extent of sperm count reduction at low doses.

Quantitative Risk Estimation: Use of Low-Dose Extrapolation and the Two-Distribution Model to Estimate Individual and Population Risks of Male Infertility

Background. The BD/UF procedure described above has one distinct advantage over the NOAEL/UF approach: It can be used to provide an estimate of risk presented by exposure to reproductive toxicants at the RfD level. The BD approach, however, does not make the estimate by modeling the entire dose range. Uncertainty factors are applied subsequent to the modeling to produce a risk estimate by assuming a linear dose-response at exposures below the BD.

It is also possible to relate a change in a specific reproductive parameter (i.e., sperm count) to a functionally and socially relevant end point (i.e., male infertility). The risk of infertility in an exposed population can be estimated by combining our low-dose extrapolation model of DBCP's effect on sperm counts with a model of the relationship between sperm count reductions and infertility risk. In this Quantitative Risk Estimation (QRE) approach, uncertainties in intra- and interspecies extrapolation and dosing regimen are addressed during the development of a potency estimate for the reproductive toxicant.

Both the BD and QRE approaches share the assumption that the relationship between DBCP dose and sperm count reduction may be approximately linear over the entire dose range, i.e., that there may be no threshold for DBCP's effects on sperm count. This assumption, which challenges conventional threshold-based approaches to reproductive risk assessment, can be justified in the case of DBCP on both biological and science policy grounds.

Available data on DBCP are inadequate to resolve conclusively whether or not there is a threshold for DBCP's toxic effects on sperm count. The doses which are associated with the reproductive toxicity of DBCP in both occupational and experimental settings are two orders of magnitude higher than current environmental exposures to DBCP in contaminated groundwater. Hypotheses about the shape of the DBCP-sperm-count dose-response curve in the low-dose region must be based on a biological model of its mechanism of action. Some proposed mechanisms of DBCP action (e.g., inhibition of epididymal sperm energy processes) might exhibit a threshold effect. However, other plausible mechanisms of action suggest it is inappropriate to rule out the possibility of low-dose linearity.

Two arguments support the biological plausibility of low-dose linearity for DBCP's effect on sperm count: *a*) It has been postulated that DNA damage is the initiating event in DBCP-induced cell death (67). The reproductive toxicity of DBCP in different animal species correlates with the extent of DNA damage (25). If DNA is the target of DBCP toxicity, it is appropriate to make the conservative assumption of low-dose linearity, as in the case of risk assessment for carcinogens. *b*) DBCP may operate by the same mechanism which is responsible for background rates of decreased sperm count production. If DBCP's effects are additive to the risks from background agents, then its dose-response curve will be approximately linear in the region of background incidence (68).

Conventional arguments in support of thresholds for noncarcinogenic toxicity may not be applicable to DBCP. The crux of most arguments is that there is an excess function available in organs, which must be exhausted before clinically significant adverse effects are observed. However, many human males do not have any excess capacity for sperm production (52,53). The reversibility of DBCP's effects on sperm count does not demonstrate a threshold in the dose-response curve. In the case of DBCP, reversibility is most clearly related to the exposure scenario. When exposure to DBCP is stopped, its effects on sperm count are reversible only if enough stem cells have survived to repopulate the seminiferous epithelium.

In the absence of any information on the distribution of individual thresholds for DBCP toxicity in the human population, we have assumed that DBCP reduces sperm counts over the entire dose range encountered in California. This no-threshold assumption is made in order to produce upper bound estimates of risk which would be useful for risk management purposes. The same assumption underlies risk estimates produced using the BD approach (3) and other proposals to establish regulatory levels for noncarcinogenic toxicants using linear extrapolation (69).

Adoption of a no-threshold assumption is ultimately a decision of science policy—it is not dictated by the toxicological data. If the conventional NOAEL/UF approach is used, the regulatory levels derived represent unknown risks of reproductive toxicity and an un-

known proportion of the population may have individual thresholds below the allowable exposure level (61). If the BD or QRE approaches are used, conservative estimates of potential reproductive risk at regulatory levels can be derived.

If one makes the plausible assumption that DBCP can reduce sperm count over the entire dose range, then there should be no threshold for its effect on fertility in an exposed population. There is a broad distribution of sperm counts in the human population, which includes individuals who are severely oligospermic and at high risk of infertility. Exposure to any agent which reduces sperm count will shift the distribution of sperm counts in the population to lower values, increasing the risk of infertility in the population (70). Reductions in sperm count may cause azoospermia in highest risk individuals and increase the risk of infertility in other individuals. This relationship between a spermatotoxicant and sperm count is illustrated in Figure 1, which displays two distributions of sperm counts. The frequency distribution of sperm counts in a group of workers exposed to DBCP is shifted to lower values when compared to the distribution of counts in the unexposed cohort. Fewer DBCP-exposed men have counts above 100 million sperm/mL, and a greater number have counts below 20 million sperm/mL, a frequently used clinical definition of infertility (18).

Risk estimates derived using the health-conservative assumption that there is no threshold for DBCP's effects on fertility represent upper bound estimates on possible risk. Actual infertility risk could be zero if individuals do have thresholds for DBCP's effect on sperm count and if no individuals in the exposed population receive doses greater than their thresholds.

Method. Meistrich and co-workers (7) have developed a method which can be used to estimate infertility risks for individuals or a population by examining changes in the frequency distribution of sperm counts. This two-distribution model for reproductive success (70) can be applied to DBCP to generate estimates of potential infertility risks in California's Central Valley.

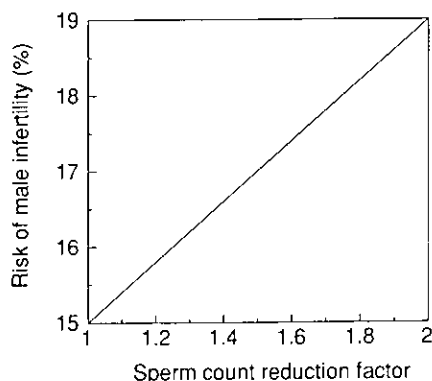


FIGURE 3. Hypothetical relationship between sperm count reduction factor and human infertility risk derived by Meistrich and Brown (7).

Evaluation of epidemiological data on the fertility of 1000 men (71) suggests that a 50% reduction in sperm counts among all members of a population produced a 4% absolute increase in the rate of male infertility in that population (i.e., infertility increases from an estimated background rate of 15 to 19%). A 10% reduction in sperm count in the human population will be associated with an absolute increased incidence of infertility of about 0.44% (i.e., infertility increases from an estimated background rate of 15 to 15.44%) (7). The relationship between sperm count reduction and infertility risk is displayed graphically in Figure 3.

Because data on human exposure are inadequate for modeling purposes, results from experimental animals were used to estimate the dose that produced a 10% reduction in sperm counts. Following the BD approach described, this dose was estimated to be 0.015 mg/kg-day in rabbits.

Uncertainties in extrapolation between species, in intraspecies variation, and in extrapolation from subchronic to chronic exposures were addressed in the EPA and BD approaches by dividing a NOAEL or BD by uncertainty factors. Some of these uncertainties are handled differently in the QRE approach, as discussed below.

Interspecies Extrapolation. INTERSPECIES SENSITIVITY TO SPERMATOTOXINS. An interspecies extrapolation factor (IEF) is needed to estimate the spermatotoxic potency of DBCP in humans from animal data. The IEF is defined (72) as a ratio:

$$\text{IEF} = \frac{\text{Dose necessary to produce a given toxic effect in test animal}}{\text{Dose necessary to produce the same effect in humans}}$$

Reduced sperm count provides a suitable end point on which to base the determination of the IEF because it is quantifiable in experimental animals and in man.

Interspecies variability in response to DBCP was addressed by assuming that humans could either be equally or more sensitive than animals and by developing potency estimates accordingly. The low estimate of an IEF for DBCP is 1, indicating that the sensitivity of humans and rabbits are equal (Fig. 4). DBCP's alkylating activity and disruption of sperm metabolism suggest that its mechanism of action in lowering sperm count may be similar in animals and man (11). Selection of an IEF of 1 is supported by a limited comparison of high-dose animal and human response to DBCP exposure. The experimental dose (0.27 mg/kg-day absorbed) that produced substantial reductions in rabbit sperm count (> 50% decrease after 14 weeks of exposure) and decreased fertility (30% decrease in total implantations per litter) was similar to the minimum dose estimate from occupational exposure (0.19 mg/kg-day absorbed) that produced substantial reductions in sperm counts and fertility in humans. Human adverse effects and dose levels were not sufficiently quantified to allow a direct calculation of a ratio of species sensitivity.

The high estimate of an IEF for DBCP (Fig. 4) assumes humans are more sensitive than rabbits. This

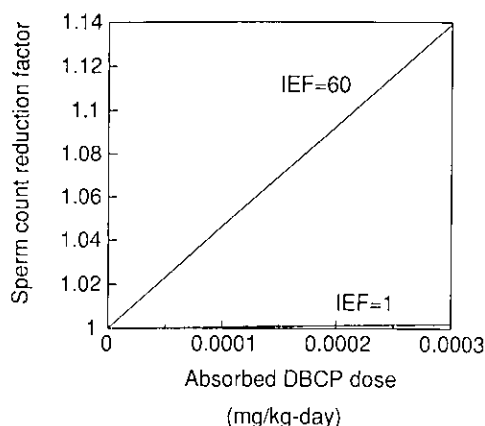


FIGURE 4. Modeled low-dose relationship between DBCP exposure at environmental levels and sperm count reduction. (IEF = 1 and IEF = 60).

estimate incorporates available data on differences in susceptibility and pharmacokinetics. Studies on the differential susceptibility of men and mice to the effect of radiation on spermatogenesis provide the only data available on interspecies sensitivity in which tissue doses are known to be the same. Similar data are not available for rabbits. In radiation exposure studies, the IEF ranges from 11 to 21 when measured at the end of the interval necessary for stem cells to become sperm (i.e., at 115 days in men and at 56 days in mice). The IEF ranges from 20 to 44 when measured at the time when ejaculate sperm counts reach a minimum (i.e., at 35 weeks in men and at 18 weeks in mice) (73). These values indicate humans are more sensitive than experimental animals to the effects of ionizing radiation on stem cells. For chemical spermatotoxins, data comparing interspecies sensitivity are sparse. A factor of 20 was selected from the data reviewed by Meistrich (71) to obtain an estimate of the potential sensitivity of human stem cells.

INTERSPECIES DIFFERENCES IN METABOLISM AND DISTRIBUTION. Administered doses are used to estimate the IEF, but they may not reflect the doses to the target organ (the testes in the case of DBCP) in different species. Such differences can be corrected for by the application of the default interspecies scaling procedure employed in cancer risk assessment, in which milligram per unit surface area is an equivalent measure of dose in different species (68,74). Scaling to the estimated human potency is obtained by multiplying the animal potency by the ratio of human to animal body weights raised to the one-third power. For 2-kg rabbits this interspecies correction factor is $(70/2)^{1/3} = 3$. An adverse effect dose in rabbits is divided by a factor of 3 to estimate a similar adverse effect dose for humans. The same scaling can be obtained by converting administered doses from milligrams per kilogram to milligrams per square meter and then deriving a potency estimate, as was done by Meistrich (7) in a similar analysis.

HIGH ESTIMATE OF THE IEF. Multiplying the available estimate of interspecies differences in sensitivity (a factor of 20) by the estimated differences in pharmacokinetics (a factor of 3) produces a high estimate of the IEF of 60. The dose producing a 10% change in rabbit sperm count is divided by 60 to estimate the dose producing a 10% change in human sperm count (Fig. 4). At an IEF of 1, there is only a minimal reduction of sperm count over the range of environmental exposures likely in California. At an IEF of 60, there is a 14% reduction in sperm count over this same range.

Intraspecies Variability. Intrahuman variability in susceptibility to the effect of sperm count reduction on fertility is incorporated into the two-distribution model. The relationship between reductions in sperm count and male infertility risk was derived (7,70) using data from 1000 men (71), providing an adequate measure of the distribution of susceptibility in a population. Intrahuman variability in susceptibility to DBCP's effect on sperm count is not explicitly addressed because of the absence of data. Uncertainties surrounding both of these sources of intraspecies variability are addressed by the use of a 10-fold uncertainty factor in the conventional NOAEL/UF approach.

Subchronic versus Chronic Exposures. The DBCP dose-response modeling is based on a rabbit study of less-than-lifetime duration. In the NOAEL and BD approaches, this limitation in the animal data was accounted for by application of a 10-fold uncertainty factor. In the QRE approach, no explicit adjustment for the subchronic duration of the rabbit study was made. As indicated by the interspecies comparison of the effects of radiation, the time at which damage is assessed influences the magnitude of the IEF: the IEF will be high if effects are assessed shortly after acute exposure or during chronic exposure, and will approach unity if effects are assessed when recovery has occurred after cessation of exposure (72).

Calculation of DBCP Potency Estimates. Low and high estimates of DBCP's potency are derived using the range of possible IEFs. The low estimate (IEF = 1) uses the potency derived from rabbit data (1.5×10^{-2} mg/kg-day)/(10% decrease in sperm count) as the human potency. A dose of 1.5×10^{-2} mg/kg-day reduces sperm count 10% and is estimated to produce a 0.44% absolute increase in infertility in an exposed population. Assuming linearity in the low-dose range, a DBCP dose of 3.4×10^{-2} mg/kg-day is estimated to produce an increase of 1% in infertility above the background rate in an exposed population.

To calculate a high estimate (IEF = 60), the rabbit potency was divided by the interspecies scaling factor of 20 and the pharmacokinetics factor of 3 to produce an estimate of the DBCP potency for humans of $(2.5 \times 10^{-4}$ mg/kg-day)/(10% decrease in sperm count). A dose of 2.5×10^{-4} mg/kg-day reduces sperm count 10% and is estimated to produce a 0.44% absolute increase in infertility in an exposed population. Assuming linearity in the low-dose range, a DBCP dose of 5.7×10^{-4} mg/kg-day is estimated to produce an increase

of 1% in infertility above the background rate in an exposed population.

Maximum Individual Risk of Infertility. The maximum DBCP dose delivered to a person drinking water from a large public water system in the Central Valley is 3.0×10^{-4} mg/kg-day (11). The low and high estimates of the maximum individual risk (MIR) of infertility are:

$$\text{Low Estimate MIR} = \frac{3.0 \times 10^{-4} \text{ mg/kg-day}}{(3.4 \times 10^{-2} \text{ mg/kg-day})/(1\% \text{ increased risk})}$$

$$= 8.8 \times 10^{-6} \text{ or approximately a 9 in 100,000 increased risk of infertility.}$$

$$\text{High Estimate MIR} = \frac{3.0 \times 10^{-4} \text{ mg/kg-day}}{(5.7 \times 10^{-4} \text{ mg/kg-day})/(1\% \text{ increased risk})}$$

$$= 5.37 \times 10^{-3} \text{ or approximately a 500 in 100,000 increased risk of infertility.}$$

The range of infertility risks arising from potential environmental exposures in the Central Valley is graphed in Figure 5. The infertility risk presented by DBCP exposure in private well water could be considerably higher. There are no data available on the magnitude of these exposures. These risk estimates are based on the health-conservative assumption that DBCP affects sperm count over the entire dose range experienced in the Central Valley. Actual risks could be zero if individuals do have thresholds for DBCP's effect on sperm count and if no individuals in the exposed population receive doses greater than their thresholds.

Aggregate Population Risk of Infertility. The aggregate population risk of increased infertility resulting from DBCP exposure in the Central Valley of California is estimated by combining the low and high estimates of DBCP potency with information on the extent of human exposures to DBCP. The doses from ingestion, inhalation, and dermal absorption of DBCP resulting from household use of contaminated water from public water systems are presented in Table 2. Estimates were made of the size of the population exposed to various doses (ranging from 2.5×10^{-6} to 3.0×10^{-4}

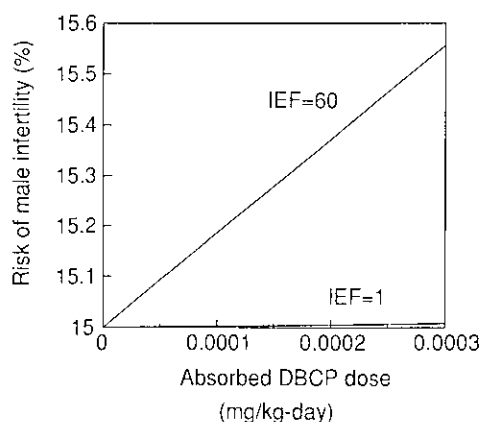


FIGURE 5. Estimated low-dose relationship between DBCP exposure at environmental levels and human infertility risk (IEF = 1 and IEF = 60).

Table 2. DBCP well water risk assessment: exposure from large public water systems in California's Central Valley (11).

Average DBCP dose per person, mg/kg-day	Number of exposed persons	Risk per group	
		IEF = 1	IEF = 60
2.5×10^{-6}	83465	0.03	1.9
7.0×10^{-6}	28093	0.03	1.8
1.2×10^{-5}	18907	0.03	2.1
1.6×10^{-5}	4506	0.01	0.7
2.2×10^{-5}	6638	0.02	1.3
2.7×10^{-5}	8687	0.03	2.1
3.2×10^{-5}	3679	0.02	1.1
3.7×10^{-5}	4448	0.02	1.5
4.0×10^{-5}	3722	0.02	1.3
4.4×10^{-5}	2377	0.02	0.9
4.9×10^{-5}	3674	0.03	1.6
5.4×10^{-5}	2782	0.02	1.3
5.9×10^{-5}	5418	0.05	2.9
6.5×10^{-5}	5522	0.05	3.2
6.9×10^{-5}	587	0.01	0.4
7.4×10^{-5}	2855	0.03	1.9
8.1×10^{-5}	3255	0.04	2.3
8.3×10^{-5}	2339	0.03	1.7
8.8×10^{-5}	2230	0.03	1.7
1.0×10^{-4}	2339	0.04	2.1
1.1×10^{-4}	2339	0.04	2.3
1.2×10^{-4}	5420	0.09	5.6
1.2×10^{-4}	1581	0.03	1.7
1.3×10^{-4}	1700	0.03	2.0
1.4×10^{-4}	1700	0.03	2.1
1.7×10^{-4}	4275	0.11	6.5
1.8×10^{-4}	732	0.02	1.2
3.0×10^{-4}	6386	0.29	17.3
Total number of excess cases of male infertility		1	70

$\times 10^{-4}$ mg/kg-day) of DBCP in contaminated drinking water (11). Assuming that the dose-response was linear over this range and that half of the exposed individuals were males, the potential minimum (IEF = 1) and maximum (IEF = 60) aggregate risks to the exposed population were calculated (Table 2).

The aggregate additional incidence of male infertility ranges between 1 and 70 cases. Infertility is defined as the number of couples trying and failing to achieve pregnancy for at least 1 year (7). Incidence estimates do not mean that the couples are permanently infertile, but that the time to pregnancy may be increased or pregnancy may not be achieved. A study of birth rates in Fresno County, CA, revealed no effects on fertility from exposures to DBCP-contaminated drinking water (75). Given the small number of infertility cases predicted by even the most conservative modeling and the limited sensitivity (76) of the measure used to assess fertility in this study (standardized birth ratio), these negative findings are not surprising.

Comparison of Reference Doses and Risk Estimates for DBCP Derived by the Four Alternative Approaches

The reference doses for reproductive toxicity derived for DBCP by the Proposition 65, EPA, and BD procedures are similar, although a slightly lower RfD was

Table 3. Regulatory levels and risk information obtainable from four approaches to reproductive risk assessment.

Approach	Regulatory level RfD ^a	Level of sperm count reduction at RfD	Risk of infertility at RfD
Proposition 65	1.9 µg/d	Not obtainable	Not obtainable
EPA	1.9 µg/d	Not obtainable	Not obtainable
Benchmark dose	1.1 µg/d	0.01%	Obtainable if association between reproductive biomarker and infertility is known
Quantitative risk assessment	Need to set acceptable risk level	Obtainable for all dose levels	Obtainable for all dose levels

^a RfD, reference dose.

derived using a BD rather than a NOAEL. The similarity of these RfD estimates does not prove that the RfD is valid; it only indicates that working from the same data set, alternative approaches produce similar regulatory levels. In the case of DBCP, alternative approaches do provide additional information on the magnitude and extent of reproductive risks that can improve both risk management and risk communication. Table 3 shows the regulatory levels derived using alternative approaches and describes the additional risk information which can be obtained from each.

Both the Proposition 65 approach and the EPA approach are based on establishing a NOAEL and dividing by conventional but arbitrary uncertainty factors. Neither approach provides information about the extent of health risk remaining at a regulatory level. In contrast, the BD approach uses all available dose-response data and associates the RfD level with a risk estimate. This approach establishes a more consistent starting point for the application of uncertainty factors and provides potentially important risk information.

The BD risk estimates, however, will generally describe a change in a reproductive parameter and not a change in a socially meaningful end point. The BD procedure can be applied to dose-response data for a variety of sensitive end points assessed in reproductive toxicity assays, but the significance of small projected alterations in these parameters (e.g., diameter of seminiferous tubules) is unclear. Only when the association between a change in a reproductive biomarker and reproductive function is known (as is the case for sperm count reduction and infertility risk) can predictions of socially meaningful risk be made. An additional disadvantage of the BD procedure is its combination of dose-response modeling and arbitrary uncertainty factors. To provide an upper bound estimate of risk, the

procedure assumes risk decreases linearly with dose from the BD estimate. This conservative assumption does not use the actual slope of the dose-response curve (derived from the experimental data) to estimate low-dose risks.

The QRE approach uses the same dose-response model of animal data as in the BD approach, but extends the model to estimate levels of sperm count reduction and risks of infertility in the low-dose region. Risks may be estimated for any point along the dose-response curve. Some of the uncertainties that are addressed with conventional 10-fold uncertainty factors can be quantitatively addressed in the development of a potency estimate in the QRE approach (e.g., variations in interspecies susceptibility and pharmacokinetics). Other uncertainties are more difficult to address in the QRE approach (e.g., subchronic to chronic exposure extrapolation), at least with the available DBCP data.

The QRE approach can be used to assess the residual reproductive risk associated with regulatory levels derived using uncertainty-factor-based approaches. At 1.9 µg/day, the RfD derived using the Proposition 65 or EPA approach, the maximum estimated risk of infertility from DBCP exposures is 4.8×10^{-4} . At 1.1 µg/day, the RfD derived using the BD approach, the maximum estimated risk of infertility from DBCP exposures is 2.8×10^{-4} .

The QRE approach also provides conservative estimates of the aggregate population incidence of male infertility produced by exposures to DBCP (Table 2). Since the contribution of various sectors of the population to total incidence can be quantified, the impact of regulatory decisions on the reduction of the number of excess cases of male infertility in the exposed population can be evaluated. Estimates of maximum individual risk and population incidence of reproductive effects are similar in concept to those produced in cancer risk assessment. Information about the magnitude of individual risks and the extent of population effects can be used to improve communication with the public about the relative importance of various reproductive hazards.

The QRE approach has several disadvantages as an alternative procedure for reproductive risk assessment. The approach can be used to derive a regulatory level only if a policy decision is made about an acceptable level of reproductive risk. But to reach social consensus on acceptable risk levels, some common unit of risk that is socially meaningful is required (comparable to the one-in-a-million lifetime cancer risk used in cancer risk assessment). Application of the QRE approach will be restricted to compounds that affect biological parameters with quantifiable impacts on reproductive success. DBCP is just one example of a number of compounds where sufficient data are available to link experimentally observed end points (reduction in sperm count) with socially significant outcomes (increased infertility in a population). Wyrobek et al. (77) catalogued 52 chemical exposures that have been re-

ported to lead to reduced sperm production in humans. The QRE approach warrants further evaluation with these agents, especially in situations that are amenable to epidemiological analyses of changes in fertility and reproductive outcome.

Models that attempt to estimate low-dose risks from exposures to reproductive toxicants challenge the traditional assumptions of reproductive risk assessment. The NOAEL/UF approach assumes there is a threshold exposure level below which no toxic effects occur, and that individuals incur a reproductive risk only if their level of exposure to an agent exceeds their individual threshold level. Due to statistical limitations on identifying a NOAEL, it can be expected in some cases that a significant proportion of the population will have individual thresholds that are below the acceptable exposure level established by the NOAEL/UF approach (61).

The linear model used in the BD and QRE approaches does not assume that there is a threshold for DBCP's toxic effects on spermatogonial stem cells or its effect on infertility in an exposed population. It is biologically plausible that stem cell loss may be linear at low doses of DBCP. If sperm counts are reduced, there will be no threshold for DBCP's effect on infertility risk. These assumptions are made in order to produce upper bound estimates of risk which would be useful for risk management and communication purposes. Using the QRE approach, reproductive risk assessment for some compounds becomes similar to cancer risk assessment and poses a fundamental question that the conventional NOAEL/UF approach ignores: What level of estimated reproductive risk is acceptable?

The selection of a preferred approach depends on the understanding of the toxicology of a substance and its effects on the population distribution of a reproductive end point as well as on a regulatory agency's legal mandate. Few precedents for regulatory decisions on reproductive or developmental toxicants are available at the State or Federal level, with California's Proposition 65 currently prominent for its emphasis on reproductive toxicity as well as cancer. The comparison of alternative approaches to deriving reference doses for reproductive toxicants provided in this risk assessment of DBCP clarifies what information and assumptions are required to replace the conventional NOAEL/UF approach.

The authors thank Marvin Meistrich for his assistance in modeling the infertility effects of DBCP. We thank K. S. Rao for sending us the original data from his 1982 inhalation study in rabbits. Statistical assistance was provided by Richard Howe of Clement International, K. S. Crump Division. We also acknowledge the helpful comments of Andrew Wyrobek and anonymous reviewers. This research was conducted with the financial support of the California Department of Health Services. This paper has been reviewed and approved for publication by the Office of Air Quality Planning and Standards, United States Environmental Protection Agency. The views in this paper are those of the authors and do not necessarily reflect the views or policies of the United States Environmental Protection Agency or the California Department of Health Services.

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